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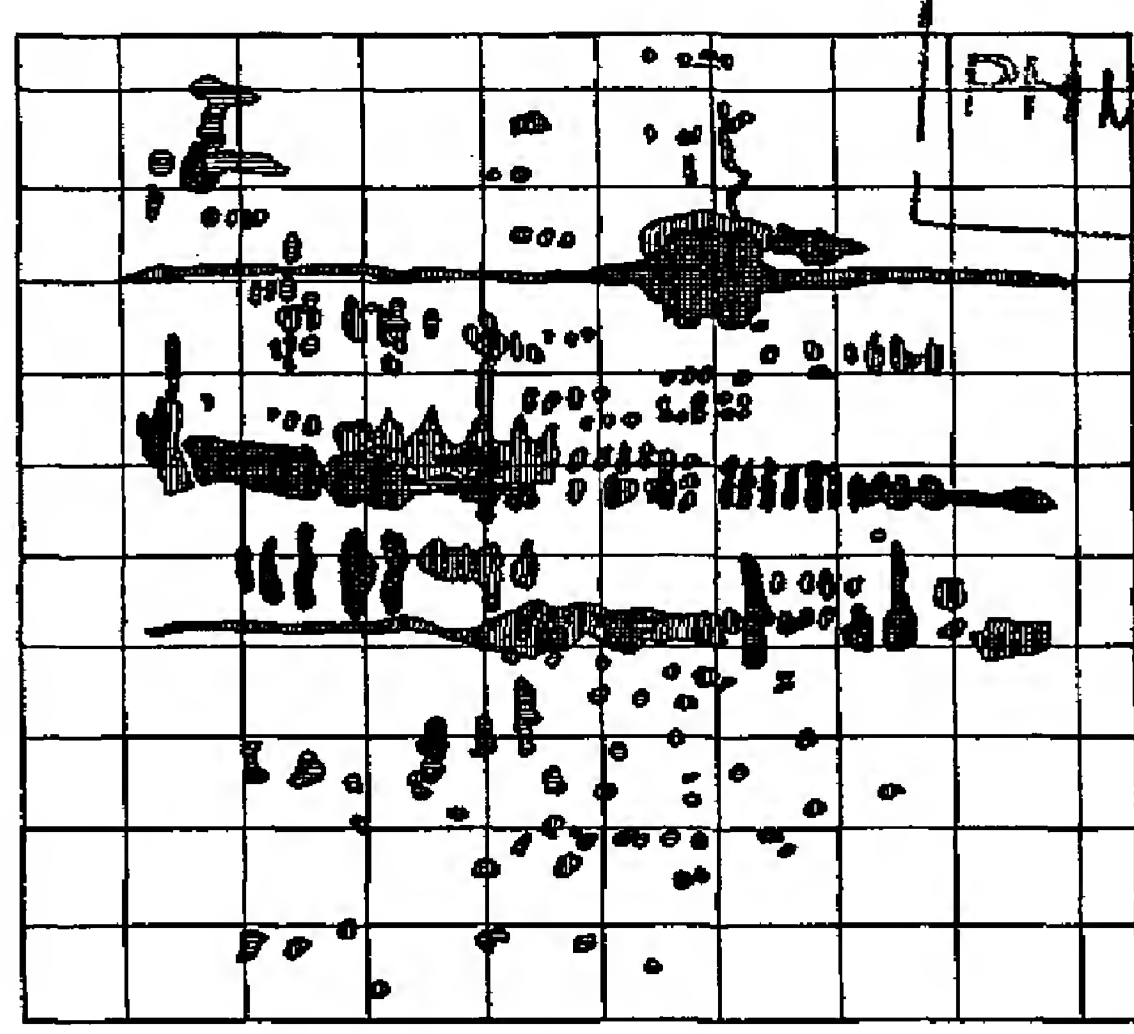

(57) **Abstract:** A method for registering first and second digital images of first and second separation patterns, respectively, where the digital images are described by gray level functions f_1 and f_2 defined on a pixel set. In accordance with the method, the pixel set is divided into m regions $R_1,...,R_k,...R_m$. For each region R_k, a sequence of n_k transformations *Sk1 Sk,j,...Sk,nk*, mapping R_k *into the* pixel set is selected. A similarity score is then calculated for each of the n_k regions $S_{k,j}(R_k)$ for $j=1,...n_k$, where the similarity score scores the similarity of the appearance of R_k in the first digital image and the appearance $S_{k,j}(R_k)$ in the second digital image. A transformation S_k is then selected from among the *Sk,1,...,Sk,j,...Sk,nk* for which the similarity score, $S_k(R_k)$ is maximal among the similarity scores of the $S_{k,j}(R_k)$ for $j=1,...n_k$. A pixel p_k in R_k is then selected. A global transformation T mapping the pixel set into the pixel set is defined based upon at least some of the $S_k(p_k)$, and the image of the second image under T^{-1}, is obtained to produce a modified second image

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METHOD FOR REGISTERING SEPARATION PATTERNS

FIELD OF THE INVENTION

The invention relates to methods of registering a pair of one- or two-dimensional separation patterns, for example such patterns as are obtained by one- or two-dimensional electrophoresis.

BACKGROUND OF THE INVENTION

Separation of a solubilized mixture of molecules into its components is routinely performed by forcing the mixture to migrate through a porous solid or fluid medium called the *"matrix"*. The matrix and other relevant factors are selected so that the various molecules of the mixture have different migration rates in the matrix according to a particular criterion. The mixture thus separates into its components as it progresses through the matrix. Conditions may be chosen so that the migration rate of a molecule will depend, for example, upon its molecular weight or its electrical charge.

At the end of the separation, the components are visualized by various methods either in the matrix or as they are eluted from the matrix, thus producing a one-dimensional separation pattern consisting of an array of spots. Each spot in the array contains all of those molecules in the mixture that migrated through the matrix at a particular rate. The molecules in a given spot may all be identical. More commonly, however, the spot will still contain a mixture of different molecules. If for example the molecules were separated according to molecular weight, then a given spot will contain all the molecules in the original mixture having about the same molecular weight.

Gel electrophoresis is a solid phase separation technique. The matrix, in this case, is a gel slab typically of polyacrylamide, and the mixture is made to migrate through the gel under the influence of an electric field. In one application, molecules such as proteins or nucleotides separate according to their molecular weight. In another application, known as isoelectric focusing, molecules separate according to their isoelectric point (the pH at which a given molecule is electrically neutral). Other solid phase separation techniques include thin layer chromatography, paper chromatography, and liquid chromatography. Other separation techniques include mass spectroscopy.

Since a given spot in a one-dimensional separation pattern may still be a mixture of different molecules, it is common to subject the spots in the pattern to a second round of separation in which the molecules are made to migrate in a direction perpendicular to that of the first round. In the second round, conditions are chosen so that the molecular components of each spot are separated according to a different criterion than was used during the first round. After the second round of separation, the molecules are visualized producing a two-dimensional separation pattern. Each spot in the pattern will contain those molecules that migrated through the matrix at the same rates during both separation rounds. In most instances, each spot will contain a single molecular species.

For example, a mixture of proteins may first be separated by gel electrophoresis according to their isoelectric point and the resulting one-dimensional separation pattern subjected to a second round of electrophoresis according to molecular weight. More than 5,000 proteins can be resolved on a single gel by this type of two-dimensional electrophoresis.

It is often of interest to compare two or more two-dimensional separation patterns. For example, a two-dimensional electrophoretogram of blood plasma proteins from two individuals may be compared. Corresponding spots in the two patterns having different sizes (indicative of a different abundance of a protein in the two samples) or spots having different locations in the patterns (indicative of a

mutated protein) are identified. Such differences may reflect the molecular basis of a disease.

In order to identify altered spots in two separation patterns, the two patterns must first be put in register. This involves identifying pairs of corresponding spots in the two patterns. Generating a one-to-one correspondence between spots in the two patterns (referred to herein as registration) is complicated by the fact that corresponding spots may have different locations and appearances in the two patterns not only due to intrinsic differences in the original samples but also due to unavoidable variability in sample preparation, matrix preparation, running conditions, visualization of the pattern and physical warping of the matrix.

Algorithms designed to register a pair of separation patterns are known in the art, for example Melanie™ and PDQuest™ marketed by Bio-Rad Laboratories and Bio Image™ marketed by Bio Image U.K. In these algorithms spots are detected in digital images of the patterns. This yields a *"spot-file"* for each pattern consisting of a list of the detected spots, their centers, boundaries and volume. The two images are then presented side by side to the user on a monitor screen and the two lists are aligned interactively. The user is prompted to indicate pairs of corresponding spots, which is often a laborious and time-consuming process.

U.S. Patent No. 5,073,963 to Sammons *et al.* discloses a computerized method for registering spots in a pair of two-dimensional gel electrophoretograms. Their method makes use of coordinate transformation techniques known in the art. A spot file for each pattern is obtained and a transformation of a predetermined type is sought that generates a one-to-one correspondence between the spots in one file and those of the other with minimal distortion of the patterns, as measured by a predetermined distortion criterion.

SUMMARY OF THE INVENTION

Within the context of the present invention, two explicitly described, calculable or measurable variables are considered equivalent to each other when the two variables are proportional to each other.

The present invention provides a method for registering digital images of two separation patterns of the same dimensionality. A pixel is a vector having the same dimensionality as the pattern. The first and second digital images are described by means of gray level function f_1 and f_2, respectively, defined on a pixel set, that describe the intensity of the image at each pixel. A region of the pixel set is a subset of the pixel set. The appearance of a region in a digital image is the restriction of its gray level function to the region.

In accordance with the invention, the registration is described by means of a transformation **T**, referred to as the global registration transformation, mapping at least a subset of the pixel set into the pixel set. This is in contrast to prior art methods where registration is performed by applying transformation techniques to the spot lists derived from the digital images. The present invention thus utilizes a larger amount of available data for the registration process than the prior art methods. The utilized data include not only the location and precise structure of the spots of the visualized molecules, but also reproducible staining in the patterns arising from other sources such as streaks and spot shapes.

In accordance with the invention, the global registration transformation **T** is obtained as follows. At least a portion of the pixel set is divided into m regions $R_1,\ldots R_k,\ldots R_m$. For each region R_k, a finite sequence of n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$ is selected, where each transformation $S_{k,j}$ maps R_k into the pixel set. This generates a sequence of n_k regions in the pixel set denoted by $S_{k,j}(R_k)$ $(j=1,\ldots n_k)$. For each of the n_k regions $S_{k,j}(R_k)$ a similarity score is calculated which scores the similarity of the appearance of R_k in the first digital image and the appearance of $S_{k,j}(R_k)$ in the second digital image. An optimal transformation S_k is selected from among the n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$ for which the similarity score of $S_k(R_k)$ is maximal.

The global transformation **T** is now determined as follows. In each region R_k, a pixel $\mathbf{p}_k$ is selected. $\mathbf{p}_k$ may be for example the center of gravity of R_k. A global transformation **T** is then found based upon at least some of the $S_k(\mathbf{p}_k)$, for $k=1,\ldots m$.

The first image may be partitioned into any number m of regions. As extreme examples, it may be partitioned into a single region, or each pixel in the image may be taken as a separate region. The regions may have any shape such as squares, rectangles, circles, ellipses or irregular shapes. The regions in a partition may all be of the same shape, or there may be regions in the partition of different shapes. The regions may or may not overlap. The image may be partitioned into a regular array of identical regions. The partition may be formed taking into account the distribution of spots in the separation pattern. For example, a partition may be formed so that no regions in the partition is devoid of spots while areas in the pattern with many will be divided into a large number of small regions.

In a preferred embodiment, the regions R_k, are chosen so that each one contains at least two spots, and most preferably three or four spots of the first digital image. If a region R_k contains too many spots, it may not be satisfactorily transformed by any of the transformations $S_{k,j}$ as assessed by the similarity scores. On the other hand, regions that are have too few spots may have a geometric signature that is not sufficiently unique, in which case several of the transformations $S_{k,j}$ may have high similarity scores. For example, a region that contains only one spot can potentially match any other single-spot region. Spots may be detected by methods known in the art. For example, the boundary of a spot in a digital image may be recognized as a location where the Laplacian of the gray level function of the pattern exceeds a threshold value. Thus, for example, the number of spots in the first digital image may be determined and the average area of a region containing four spots calculated. A region of this area is constructed around every spot in the first pattern, and a score calculated for each of the regions that takes into account the number of spots in the region, their contrast and area. For each pair of strongly overlapping regions, the one with the lowest score is discarded.

In accordance with another embodiment, regions in the pixel set are first designated and then ordered. For example, a score is calculated for each of the regions that takes into account the number of spots in the region in the first image,

their contrast and area. A region R_1 is selected as the region having a high score near the center of the first digital image. The remaining regions are then sequentially ordered according to increasing distance from the first region.

In yet another embodiment, the transformations $S_{k,j}$ are translations of the form $S_{k,j}(x)=x+a_{k,j}$, where $a_{k,j}$ are translation vectors. The translation vectors may be selected, for example, among lattice points of the form $l\mathbf{s}$, for one-dimensional digital images, and $l\mathbf{s}_1+h\mathbf{s}_2$ for two-dimensional images, where **s**, $\mathbf{s}_1$ and $\mathbf{s}_2$ are vectors, $\mathbf{s}_1$ and $\mathbf{s}_2$ are linearly independent, and l an h are integers satisfying $|l|<d_1$ and $|h|<d_2$ for predetermined constants d_1 and d_2. The vector **s** or the vectors $\mathbf{s}_1$ and $\mathbf{s}_2$ are most preferably chosen such that their magnitudes are approximately equal to the radius of the smallest expected spot in the digital image and will typically be around 3 pixels. Other types of transformations $S_{k,j}$ are also envisaged within the scope of the invention including linear transformations and affine transformations. The transformations $S_{k,j}$ may take into account torn parts or warping of the matrix, for example by using models of deformable membranes as disclosed, for example, in Singh et al., 1998.

The similarity score is designed to take into account differential expression, different staining qualities or techniques, and other irregularities in the two images such as artefactual staining, contamination, air bubbles etc. The scoring function compares the appearance of a region R_k in the first image to that of each of the n_k regions $S_{k,j},(R_k)$ in the second image. The scoring functions thus use nearly all of the information contained in the two images. This is in strong contrast with prior art registration methods that use only the list of approximated spot profiles, or Gaussians of the images.

The similarity score of R_k in the first image and $S_{k,j}(R_k)$ in the second image may be described as a function having, for example, the form $\Sigma G(\mathbf{x}, S_{k,j}(\mathbf{x}))$ where the sum is taken over all $x \in R_k$. G is a function that preferably increases if the contrast at **x** and $S_{k,j}(\mathbf{x})$, in the first and second images, respectively, both exceed a predetermined minimal contrast. G may be further increased, for example, when the contrast at **x** and $S_{k,j}(\mathbf{x})$ are both above a second higher predetermined level. This

favors matching of strong spots over matching of faint spots or spots with dissimilar contrast. The score may also take into account a comparison of the scalar product of the two intensity gradient vectors. This gives an added bonus for spots that have about the same size and shape. This contribution arises from the edge pixels of the spots. In cases where the edges overlap and are similarly oriented, the score is increased. Where there is no correlation or negative correlation, it diminishes. This contribution mimics some of the processing that the human eye does when attempting to match gel images. It is reasonable to prefer a transformation that matches spots with similar contrast, size and shape, to one that matches spots that differ widely.

The selection of the optimal transformations $S_1,\ldots,S_k,\ldots,S_m$ may be performed iteratively. For example, once the optimal transformations $S_1,\ldots,S_{k-1}$ have been selected, a similarity score may be used that prefers an S_k that is similar to one or more of any of the $S_1,\ldots,S_{k-1}$.

The global transformation **T** may be obtained from the $\mathbf{S_k(p_k)}$ by several different methods as are known in the art. If the number of regions (m) is 1, 2 or 3, **T** may be defined as the unique translation, linear transformation, or affine transformation, respectively, determined by the constraints $\mathbf{T(p_k)=S_k(p_k)}$, for k=1,...m. Even when m is large, a translation may be satisfactory, for example, if the two images were obtained from different scans of the same separation pattern. If the images are from different patterns, but the separation technique is known to have very tight geometric tolerances (for example, by using a matrix having a rigid backing), then an affine transformation may be satisfactory. An affine transformation compensates for shift, rotation, scaling, and skew of the axes, but does not compensate for other types of deformation such as warping of the matrix. In cases such as these, **T** may be a radial basis function transformation, for example, as disclosed in Poggio and Girosi 1989, Poggio and Girosi 1990, or Girosi *et al. 1995*, or a Delaunay triangulation transformation, as disclosed, for example, in Kanaganathan *et al.* 1991, or Baker 1987. Once the global

transformation has been found, the image can be transformed in one of several standard known ways such as nearest neighbor, bilinear, or bicubic interpolation.

Once a global transformation function has been obtained in accordance with the invention, it may be revised, for example, using optical flow equations, as disclosed, for example in Aggarwal *et al.* 1988, or Huang *et al.*, 1994. In this method, a desired correction vector w(**x**) is found by minimizing the expression $\sum(\nabla g \cdot w - dg)^2$, wherein ∇g is the gradient vector of the intensity at **x** and dg is the gray level difference between **x** and T(**x**). The minimum is easily found using least mean square fitting techniques for example as disclosed in Press *et al., 1992*. **T**(**x**) is then replaced with **T**(**x**)+w(**x**). This revision improves robustness and precision and may be repeated several times.

The results of the registration may be presented to a user by superimposing the two digital images on a monitor screen such that a pixel in one digital image is superimposed upon its image under the transformation in the other. The two digital images may be false-colored. If, for example, the spots in one image are false colored blue, and those in the other digital image are false-colored yellow, then any superimposed spots in the superimposition will appear green.

The invention may be used to compare each of several separation patterns to one particular separation pattern referred to as the "*master pattern*". The master pattern may be annotated by compiling information on the composition of each spot in the pattern. For example, a two-dimensional electrophoretogram of blood plasma proteins from a healthy individual may be prepared and used as a master pattern. Test electrophoretograms from other individuals are then registered with the master pattern. When the digital image of a test pattern that has been registered with the master pattern is then viewed on a monitor screen, a spot in the test pattern may be selected, and the compiled information on the spot presented to the user.

The invention may be also be used to follow time dependent changes in molecular composition. For example, samples of blood plasma of proteins from an individual undergoing medical treatment may be obtained at various times and a two-dimensional separation pattern of each sample prepared. Each separation

pattern may be registered with a master pattern or the previous pattern in the sequence. The registered patterns may then be displayed sequentially in time on a monitor screen so as to animate time dependent changes in the composition of the samples.

It will also be understood that the invention contemplates a computer program being readable by a computer for executing the method of the invention. The invention further contemplates a machine-readable memory tangibly embodying a program of instructions executable by the machine for executing the method of the invention.

Thus, in its first aspect the invention provides a method for registering first and second digital images of first and second separation patterns, respectively, the digital images being described by gray level functions f_1 and f_2, respectively, defined on a pixel set, the method comprising the steps of:

a dividing at least a portion of the pixel set into m regions $R_1,\ldots,R_k,\ldots R_m$, the m regions each having an appearance in the first image;

b For each region R_k,

- **ba** selecting a finite sequence of n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$, each transformation mapping R_k into the pixel set;
- **bb** generating n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$ in the pixel set, the n_k regions each having an appearance in the second digital image;
- **bc** calculating a similarity score for each of the n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$, the similarity score scoring the similarity of the appearance of R_k in the first digital image and the appearance $S_{k,j}(R_k)$ in the second digital image;
- **bd** selecting a transformation S_k from among the n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$ for which the similarity score of $S_k(R_k)$ is maximal among the similarity scores of the $S_{k,j}(R_k)$ for $j=1,\ldots n_k$; and

be selecting a pixel $\mathbf{p}_k$ in R_k;

c defining a global transformation **T** mapping at least a portion of the pixel set into the pixel set based upon at least some of the $S_k(\mathbf{p}_k)$; the transformation **T** having an inverse $\mathbf{T}^{-1}$, the second image having an image under $\mathbf{T}^{-1}$; and

d obtaining the image of the second image under $\mathbf{T}^{-1}$, to produce a modified second image.

In its second aspect, the invention provides a program storage device readable by machine tangibly embodying a program of instructions executable by the machine to perform method steps for registering first and second digital images of first and second separation patterns, respectively, the digital images being described by gray level functions f_1 and f_2, respectively, defined on a pixel set, the registering comprising the steps of:

a dividing at least a portion of the pixel set into m regions $R_1,\ldots,R_k,\ldots R_m$, the m regions each having an appearance in the first image;

b For each region R_k,

ba selecting a finite sequence of n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,m}$, each transformation mapping R_k into the pixel set;

bb generating n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$ in the pixel set, the n_k regions each having an appearance in the second image;

bc calculating a similarity score for each of the n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$, the similarity score scoring the similarity of the appearance of R_k in the first image and the appearance $S_{k,j}(R_k)$ in the second image; and

bd selecting a transformation S_k from among the n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,m}$ for which the similarity score of $S_k(R_k)$ is maximal among the similarity scores of the $S_{k,j}(R_k)$ for $j=1,\ldots n_k$;

be selecting a pixel $\mathbf{p}_k$ in R_k;

c defining a global transformation **T** mapping at least a portion of the pixel set into the pixel set based upon at least some of the $S_k(\mathbf{p}_k)$; the

transformation T having an inverseT-1, the second digital image having an image under T-1; and

d obtaining image of the second image under $\mathbf{T}^{-1}$, to produce a modified second image.

In its third aspect, the invention provides a computer program product comprising a computer useable medium having computer readable program code embodied therein for registering first and second digital images of first and second separation patterns, respectively, the digital images being described by gray level functions f_1 and f_2, respectively, defined on a pixel set, the registering comprising the steps of:

a dividing at least a portion of the pixel set into m regions $R_1,\ldots,R_k,\ldots R_m$, the m regions each having an appearance in the first image;

b For each region R_k,

ba selecting a finite sequence of n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,nk}$, each transformation mapping R_k into the pixel set;

bb generating n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$ in the second digital image, n_k regions each having an appearance in the second image;

bc calculating a similarity score for each of the n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$, the similarity score scoring the similarity of the appearance of R_k in the first image and the appearance of $S_{k,j}(R_k)$ in the second image;

bd selecting a transformation S_k from among the n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,m}$ for which the similarity score of $S_k(R_k)$ is maximal among the similarity scores of the $S_{k,j}(R_k)$ for $j=1,\ldots n_k$;

c defining a global transformation $\mathbf{T}$ mapping at least a portion of the pixels in the pixel set into the pixel set based upon at least some of the $S_k(\mathbf{p}_k)$; the transformation $\mathbf{T}$ having an inverse $\mathbf{T}^{-1}$, the second digital image having an image under $\mathbf{T}^{-1}$; and

d obtaining the image of the second image under $\mathbf{T}^{-1}$, to produce a modified second image.

In its fourth aspect, the invention provides a program storage device readable by machine, tangibly embodying a program of instructions executable by the machine to perform method steps for registering a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising the steps of:

a registering the digital images I_1 and I_2 according to any one of Claims 1 to 15; to produce an image I'_2 that is the modified image of I_2;

b registering I'_k and I_{k+1}, for all k from 2 to n-1 according to any one of Claims 1 to 15 to produce an image I'_{k+1}, where I'_k and I'_{k+1} are the modified images of I_k and I_{k+1}, respectively.

In its fifth aspect, the invention provides a computer program product comprising a computer useable medium having computer readable program code embodied therein for registering a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the computer program product comprising:

a computer readable program code for causing the computer to register the digital images I_1 and I_2 according to any one of Claims 1 to 15; to produce an image I'_2 that is the modified image of I_2;

b computer readable program code for causing the computer to register I'_k and I_{k+1}, for all k from 2 to n-1 according to any one of Claims 1 to 15 to produce an image I'_{k+1}, where I'_k and I'_{k+1} are the modified images of I_k and I_{k+1}, respectively.

In its sixth aspect, the invention provides a program storage device readable by machine, tangibly embodying a program of instructions executable by the machine to perform method steps for registering a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising registering a master pattern and the image I_k according to any one of Claims 1 to 15 so as to produce an image I'_k, where I'_k is the modified image of I_k, for all k from 1 to n.

In its seventh aspect, the invention provides a program storage device readable by machine, tangibly embodying a program of instructions executable by the machine to perform method steps for displaying a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising:

- **a** registering the sequence of images according to Claim 32, so as to produce a second sequence of images I'_1, $I'_2\ldots I'_n$, where I'_1 is the image I_1 and I'_k is the modified image of I_k, for k from 2 to n; and
- **b** displaying the second sequence of images $I'_1,\ldots I'_n$ sequentially on a display.

In its eighth aspect, the invention provides a computer program product comprising a computer useable medium having computer readable program code embodied therein for displaying a sequence of n digital images $I_n,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the computer program product comprising:

- **a** computer readable program code for causing the computer to register the sequence of images according to Claim 32, so as to produce a second sequence of images I'_1, $I'_2\ldots I'_n$, where I'_1 is the image I_1 and I'_k is the modified image of I_k, for k from 2 to n; and
- **b** computer readable program code for causing the computer to display the second sequence of images $I'_1,\ldots I'_n$ sequentially on a display.

In its ninth aspect, the invention provides, a program storage device readable by machine, tangibly embodying a program of instructions executable by the machine to perform method steps for displaying a sequence of n digital images $I_n,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising:

- **a** registering the sequence of images according to Claim 33 so as to produce a second sequence of images I'_1, $I'_2,\ldots I'_n$, where I'_k is the modified image of I_k; and
- **b** displaying the second sequence of images $I'_1,\ldots I'_n$ sequentially on a display.

In its tenth aspect, the invention provides a computer program product comprising a computer useable medium having computer readable program code embodied therein for displaying a sequence of n digital images $I_n,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the computer program product comprising:

a computer readable program code for causing the computer to register the sequence of images according to Claim 33 so as to produce a second sequence of images $I'_1, I'_2,\ldots I'_n$, where I'_k is the modified image of I_k; and

b computer readable program code for causing the computer to display the second sequence of images $I'_1,\ldots I'_n$ sequentially on a display.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, preferred embodiments will now be described, by way of non-limiting examples only, with reference to the accompanying drawings, in which:

Figs. 1a and **1b** show a digital image of a first and second two-dimensional separation pattern, respectively, Fig. **1d** shows a superimposition of the patterns shown in Figs. **1a** and **1b**, and Fig. **1c** shows the registration of the two images according to one embodiment of the invention;

Figs. 2a and **2b** show a digital image of a first and second two-dimensional separation pattern, respectively, Fig. **2d** shows a superimposition of the patterns shown in Figs. **2a** and 2**b**, and Fig. **2c** shows the registration of the two images according to a second embodiment of the invention; and

Figs. 3a and **3b** show a digital image of a first and second two-dimensional separation pattern, respectively, Fig. **3d** shows a superimposition of the patterns shown in Figs. **3a** and **3b**, and Fig. **3c** shows the registration of the two images according to a third embodiment of the invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

First Embodiment: Global Affine Transformation Model

The first embodiment will be demonstrated in relation to a pair of two-dimensional images. The pixel set is partitioned into a single region (the entire pixel set) and the identity transformation is used as the local transformation S_1. The global transformation **T** is an affine transformation of the form

$$T(x_1,x_2)=\begin{pmatrix} a_{1,1} & a_{1,2} \\ a_{2,1} & a_{2,2} \end{pmatrix}\begin{pmatrix} x_1 \\ x_2 \end{pmatrix}+\begin{pmatrix} b_1 \\ b_2 \end{pmatrix}$$

T is determined as follows. Let $\mathbf{x}^1,\ldots\mathbf{x}^n$ be the n pixels in the pixel set, where $x^k=(x_1^k,x_2^k)$. Define $\mathbf{T}_0$ as the identity transformation on the pixel set, and define the gray level function g_0 by $g_0(\mathbf{x}^k)=f_1(\mathbf{x}^k)$, for every pixel $\mathbf{x}^k$ in the pixel set, where f_1 is the gray level function of the first image. Once $\mathbf{T}_i$ and g_i have been found, $\mathbf{T}_{i+1}$ and g_{i+1} are found as follows. Denote by $\nabla(\mathbf{x}^k)=(\nabla_1(\mathbf{x}^k),\nabla_2(\mathbf{x}^k))=(\nabla(g_i(\mathbf{x}^k))+\nabla(f_2(\mathbf{x}^k)))/2$ the average gradient vector of the two gray level functions g_i and f_2, where f_2 is the gray level function of the second image. $\mathbf{T}_{i+1}$ is an affine transformation whose six parameters $a_{1,1}$, $a_{1,2}$, $a_{2,1}$, $a_{2,2}$, b_1, and b_2 are found by solving the following overdetermined system of linear equations:

$$\begin{pmatrix} x_1^1\nabla_1(x^1) & x_2^1\nabla_1(x^1) & x_1^1\nabla_2(x^1) & x_2^1\nabla_2(x^1) & \nabla_1(x^1) & \nabla_2(x^1) \\ x_1^2\nabla_1(x^2) & x_2^2\nabla_1(x^2) & x_1^2\nabla_2(x^2) & x_2^2\nabla_2(x^2) & \nabla_1(x^2) & \nabla_2(x^2) \\ & & & \cdots & & \\ x_1^n\nabla_1(x^n) & x_2^n\nabla_1(x^n) & x_1^n\nabla_2(x^n) & x_2^n\nabla_2(x^n) & \nabla_1(x^n) & \nabla_1(x^n) \end{pmatrix}\cdot\begin{pmatrix} a_{11} \\ a_{12} \\ a_{21} \\ a_{22} \\ b_1 \\ b_2 \end{pmatrix}=$$

$$\begin{pmatrix} g_i(x^1)-f_2(x^1) \\ g_i(x^2)-f_2(x^2) \\ \cdots \\ g_i(x^n)-f_2(x^n) \end{pmatrix}$$

g_{i+1} is then defined by $g_{i+1}(\mathbf{x}^k)=g_i(\mathbf{T}_i(\mathbf{x}^k))$. A transformation $\mathbf{A}_i$ is defined as the composition of $\mathbf{T}_1$ to $\mathbf{T}_i$, $\mathbf{A}_i=\mathbf{T}_i?\,\mathbf{T}_{i-1}?\ldots?\mathbf{T}_1$. The process is terminated when an $\mathbf{A}_i$ is obtained for which the fraction of pixels $\mathbf{x}^k$ in the pixel set for which the distance between $\mathbf{x}^k$ and $\mathbf{A}_i(\mathbf{x}^k)$ exceeds a predetermined distance (for example 10 pixels) is small (for example, less than 10% of all of the pixels in the pixel set). The global transformation $\mathbf{T}$ is then defined as $\mathbf{T}=\mathbf{A}_i$.

If at any stage, the transformation $\mathbf{T}_i$ is large in the sense that regions of the first image are moved large distances in the second image, then the images may be preliminarily blurred and decimated. This may be performed by methods known in the art for example as described in E. Adelson, *et al.* 1984. The preferred way of blurring and decimating the image is to average every 3X3 subarray of pixels of the images into one pixel of the decimated/blurred image. The image can then be blurred further, by a binomial operator of the form $\begin{pmatrix}1 & 2 & 1\\ 2 & 4 & 2\\ 1 & 2 & 1\end{pmatrix}/16$. $\mathbf{T}_i$ may then be modified by replacing b_1 and b_2 with $3b_1$ and $3b_2$.

Figs. 1a and 2a schematically show first and second digital images, respectively, of two-dimensional electrophoretograms of urine proteins. The proteins were first separated in the horizontal direction according to their isoelectic point and subsequently in the vertical direction according to their molecular weight. In Figs. 1a and 1b, each spot is represented schematically by a black curve delineating its boundary. The interior of each spot is indicated by hatching that is vertical in Fig. 1a and horizontal in Fig. 1b. Fig. 1d shows a superimposition of the images. A global transformation $\mathbf{T}$ between the two separation patterns was obtained in accordance with this embodiment and is shown in Fig. 1c. In Fig. 1c, the appearance of a pixel $\mathbf{x}$ in the first digital image has been superimposed on the appearance of its image $\mathbf{T}(\mathbf{x})$ in the second image. Regions of overlap of spots in the two images appear in Figs. 1c and 1d by vertical hatching superimposed on horizontal hatching. The amount of overlap is substantially greater in Fig. 1c compared to Fig. 1d.

Second Embodiment: Two Dimensional Spline Transformation.

The second embodiment will be demonstrated in relation to a pair of two-dimensional images. The pixel set is partitioned into a grid of rectangles R_k, all having the same dimensions whose centers lie on a lattice spanned by two linearly independent vectors s_1 and s_2. The local transformations S_k are translations and the global transformation **T** is a spline.

The width w and height h of the rectangles, can be predetermined constants or determined heuristically in each case. For example, a spot of diameter d (in pixels) in the first image may be selected and the parameters obtained by $w = h = |s_1| = |s_2| = 10d$.

Background contrast images (BCI) B_1 and B_2 are calculated for the first and second images, respectively. The BCI is a function of the pixels in an image the value of which is 0 for pixels not belonging to a spot. For pixels in a spot, the value of the BCI is a reflection of the contrast of the spot. For two pixels **x** and **y**, the pixel score function $Psf(x,y)$ is defined as follows: if $B_1(\mathbf{x})$ or $B_2(\mathbf{y})$ is zero, then $Psf(x,y) = 0$. Otherwise, **x** is in a spot in the first image and **y** is in a spot in the second image, and

$$Psf(x,y) = 1 + \alpha \min(B_1(x), B_2(y)) + \beta \langle \nabla(f_1(x)), \nabla(f_2(y)) \rangle^{\frac{1}{2}},$$

where $\langle \nabla(f_1(x)), \nabla(f_2(y)) \rangle$ indicates the absolute value of the scalar product of the two gradient vectors $\nabla(f_1(x))$ and $\nabla(f_2(y))$. This formula allows a fixed contribution (1) to the score if **x** and **y** both belong to a spot. This allows spots representing differently expressed proteins to match. There is also a "bonus" for cases where both spots have high contrast, by increasing the score in correlation with the minimum of the two contrast values. This encourages matching of strong spots over matching of faint spots or spots with dissimilar contrast. There is also a contribution from the scalar product of the two gradient vectors. This gives an added bonus for spots that have the same size and shape.

Where there is no correlation or negative correlation, the score is low. This mimics some of the processing that the human eye does when attempting to match images. The constants α, β are weighting factors that determine the relative contribution of the three summands in the definition of *Psf*. α will typically be set to the value of the minimal contrast required for a spot. Typical values of α and β are $\alpha = \beta = 0.1$.

The local transformations $S_{k,j}$ are translations of the form $S_{k,j}(\mathbf{x})=\mathbf{x}+\boldsymbol{a}_{k,j}$, for $j=1,\ldots n_k$. The $\boldsymbol{a}_{k,j}$ are selected so that the centers of the regions $S_{k,j}(R_k)$ in the second image form a lattice with a typical distance of s pixels between neighbors, where s is the radius of the smallest expected spot on the gel. This decreases the computational load by s^2 (typically, s=3). The $\boldsymbol{a}_{k,j}$ are also selected so that the regions $S_{k,j}(R_k)$ cover an area large enough that $S_k(R_k)$ lies far from the boundary of the union of all of the regions $S_{k,j}(R_k)$. The score function for $S_{k,j}$ is the sum of the *psf*($\mathbf{x}$, $S_{k,j}(\mathbf{x})$) for all $\mathbf{x}$ in R_k. An $S_{k,j}$ having maximal score is then selected and designated as S_k. As in the first embodiment, this selection of S_k may be refined, for example, by optical flow techniques as disclosed, for example, in Aggarwal *et al*., 1988, Huang *et al*. 1994. Alternatively, the S_k may be refined taking into account the scores of rectangles in the neighborhood of the selected one and determining optimal shift. Once an S_k is obtained, it may be checked. For example, if it is on the boundary of the original search area, or its score is below a predetermined threshold, or it deviates substantially from its nearest neighbors, it may be discarded.

The global transformation in this embodiment is a two dimensional spline calculated from the transformations S_k by methods known in the art, for example as disclosed in de Boor, 1978. Since the $\boldsymbol{a}_{k,j}$ are computed along a grid, the spline can be computed first on the horizontal lines, and than on the vertical lines.

Figs. 2a and 2b schematically show first and second digital images, respectively, of two-dimensional electrophoretograms of urine proteins. The proteins were first separated in the horizontal direction according to their

isoelectic point and subsequently in the vertical direction according to their molecular weight. In Figs. 2a and 2b, each spot is represented schematically by a black curve delineating its boundary. The interior of each spot is indicated by hatching that is vertical in Fig. 2a and horizontal in Fig. 2b. Fig 2d shows a superimposition of the images. The first separation pattern has been divided into rectangles whose centers lie on a lattice. A global transformation **T** between the two images was obtained in accordance with this embodiment and is shown in Fig. 2c. In Fig. 2c, a pixel **x** in the first digital image has been superimposed on its image **T**(**x**) in the second image. Fig. 2c also shows the system of rectangles that was used in this example. Regions of overlap of spots in the two images appear in Figs. 2c and 2d by vertical hatching superimposed on horizontal hatching. The amount of overlap is substantially greater in Fig. 2c compared to Fig. 2d.

Third Embodiment: Delaunay Triangulation Transformation Model

The third embodiment will now be described in relation to a pair of two-dimensional images. As in the second embodiment, in this embodiment the pixel set is partitioned into rectangles R_k. However, the rectangles are selected in accordance with the data and do not necessarily form a grid. The local transformations $S_{k,i}$ are translations and the global transformation **T** is a spline.

The rectangle sequence determination proceeds as follows: A spot list is first prepared of the first image by methods known in the art, for example as described in Gonzalez et al., 1977 or Pratt, 1991. The average area S of a rectangle (in pixels) containing four spots is $S = 4A/n$, where A is the number of pixels in the pixel set, and n is the total number of spots in the image. A rectangle is constructed with area A around every spot. In each rectangle, all spots are found with contrast above a given threshold. A score s is calculated for the rectangle by $s = a[n]\cdot c$, where n is the number of spots in the rectangle, c is the average contrast of the spots, and a[n] is a weighting function that prefers rectangles containing a number of spots close to predetermined number. In a

preferred embodiment, rectangles are preferred in which the number of spots is around 3. In this case, a can be defined, for example, by *0,10,15,20, 15, 12, 10, 8, 7, 6, 5, 4, 3, 2, 1,* for n equal to 0 to 14, and a[n]=0 for n greater than 14. For each pair of strongly overlapping rectangles, the one with the higher score is retained and the one with the lower score is deleted.

The rectangles are now ordered. A rectangle R_1 with a high score is found near the center of the image. The remaining rectangles are subsequently ordered by increasing distance from R_1.

The global transformation in this embodiment is an affine transformation and a Delaunay interpolation triangulation transformation. The affine transformation is obtained as in the first embodiment, and then the Delaunay interpolation is used for the differences between the S_k and the affine transformation. This provides a stable and robust transformation that is easy extrapolated, as well as a method for dealing with relatively small and non-uniform perturbations.

The global transformation in each of the R_k is obtained in a process similar to that of the first embodiment. However, in this embodiment, the global transformation is recomputed after each S_k has been found. Since initially there may be insufficient parameters to compute the affine transformation, simpler transformations are computed in the first three steps (identity, translation, linear transformation). At each stage, S_k is used in obtaining the shift vector $\boldsymbol{a}_{k+1}$ of S_{k+1}

A Delaunay triangulation is obtained by methods known in the art, for example, as described in Kanaganathan *et al.*, 1991. The Delaunay triangulation is used to interpolate the global transformation **T** in the convex hull of the triangulation vertices. At the four corners of the image, the Delaunay triangulation is set to zero so that the corners of the image, the global transformation **T** is affine only.

Figs. 3a and 3b schematically show first and second digital images, respectively, of two-dimensional electrophoretograms of urine proteins. The proteins were first separated in the horizontal direction according to their isoelectic point and subsequently in the vertical direction according to their

molecular weight. In Figs. 3a and 3b, each spot is represented schematically by a black curve delineating its boundary. Fig. 3d shows a superimposition of the images. The interior of each spot is indicated by hatching that is vertical in Fig. 3a and horizontal in Fig. 3b. The pixel set was divided into rectangles of different sizes so that each rectangle has about the same number of spots. A global transformation **T** was obtained in accordance with this embodiment and is shown in Fig. 3c. In Fig. 3c, the appearance of a pixel **x** in the first digital image has been superimposed on the appearance of its image **T(x)** in the second image. Fig. 3 also shows the system of rectangles that was used in this example. Regions of overlap of spots in the two images appear in Figs. 3c and 3d by vertical hatching superimposed on horizontal hatching. The amount of overlaps is substantially greater in Fig. 3c compared to 3d.

In the method claims that follow, alphabetic characters used to designate claim steps are provided for convenience only and do not imply any particular order of performing the steps.

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CLAIMS:

1. A method for registering first and second digital images of first and second separation patterns, respectively, the digital images being described by gray level functions f_1 and f_2, respectively, defined on a pixel set, the method comprising the steps of:

a dividing at least a portion of the pixel set into m regions $R_1,\ldots,R_k,\ldots R_m$, the m regions each having an appearance in the first image;

b For each region R_k,

ba selecting a finite sequence of n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,m}$, each transformation mapping R_k into the pixel set;

bb generating n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$ in the pixel set, the n_k regions each having an appearance in the second digital image;

bc calculating a similarity score for each of the n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$, the similarity score scoring the similarity of the appearance of R_k in the first digital image and the appearance $S_{k,j}(R_k)$ in the second digital image;

bd selecting a transformation S_k from among the n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$ for which the similarity score of $S_k(R_k)$ is maximal among the similarity scores of the $S_{k,j}(R_k)$ for $j=1,\ldots n_k$; and

be selecting a pixel $\mathbf{p}_k$ in R_k;

c defining a global transformation **T** mapping at least a portion of the pixel set into the pixel set based upon at least some of the $S_k(\mathbf{p}_k)$, the transformation **T** having an inverse $\mathbf{T}^{-1}$, the second digital image having an image under $\mathbf{T}^{-1}$; and

d obtaining the image of the second image under $\mathbf{T}^{-1}$, to produce a modified second image.

2. The method of Claim 1 wherein the first and second separation patterns are one-dimensional separation patterns.

3. The method of Claim 1 wherein the first and second separation patterns are two-dimensional separation patterns.

4. The method of any one of the previous claims wherein the first and second separation patterns are obtained using a separation technique selected from the list comprising:

(a) Electrophoresis;

(b) paper chromatography;

(c) thin-layer chromatography;

(d) high pressure liquid chromatography; and

(e) mass spectroscopy.

5. The method of any one of the previous claims wherein the number of regions, m, is equal to one.

6. The method of any one of the previous claims wherein one or more of the regions R_k contain exactly one pixel.

7. The method of any one of the previous claims wherein one or more of the regions R_k are rectangles.

8. The method of any one of the previous claims wherein the regions R_k are rectangles that are regularly arranged in the first image.

9. The method of any one of the previous claims wherein the step of dividing at least a portion of the pixel set into m regions R_k for k=1 to k=m comprises the steps of designating a scoring function, the scoring function scoring regions in the pixel set and selecting regions R_k for k=1 to m, where each region has a score greater than a predetermined value.

10. The method of Claim 9 wherein the scoring function comprises the algorithmic expression $s=a[n]\cdot c$, wherein n is the number of spots in a region, c is the average contrast of the spots and $a[n]$ is a function of the number of spots in the region.

11. The method of Claim 10 wherein $a[n]$ has a maximum at n about equal to 3.

12. The method of any one of the previous claims wherein one or more of the transformations $S_{k,j}$ are of a type selected from the list comprising:

(a) translations;

(b) linear transformations; and

(c) affine transformations.

13. The method of any one of the previous claims wherein the similarity score scoring the similarity of the appearance of R_k in the first image and the appearance of $S_{k,j}(R_k)$ in the second image is given by the arithmetic expression Σ *psf*(**x**, $S_{k,j}$(**x**)), wherein the sum is taken over all **x** in R_k, and $Psf(x,y) = 1 + \alpha \min(B_1(x), B_2(y) + \beta\langle \nabla(f_1(x)), \nabla(f_2(y))\rangle^{1/2}$ for any two pixels **x** and **y**, wherein B_1 and B_2 are background contrast images of the first and second digital images, respectively, and α and β are two predetermined constants.

14. The method of any one of the previous claims wherein the global transformation is a spline.

15. The method of any one of Claims 1 to 14 wherein the global transformation in a Delaunay triangulation transformation.

16. A program storage device readable by machine tangibly embodying a program of instructions executable by the machine to perform method steps for registering first and second digital images of first and second separation patterns, respectively, the digital images being described by gray level functions f_1 and f_2, respectively, defined on a pixel set, the registering comprising the steps of:

a dividing at least a portion of the pixel set into m regions $R_1,\ldots,R_k,\ldots R_m$, the m regions each having an appearance in the first image;

b For each region R_k,

ba selecting a finite sequence of n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$, each transformation mapping R_k into the pixel set;

bb generating n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$ in the pixel set, the n_k regions each having an appearance in the second image;

bc calculating a similarity score for each of the n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$, the similarity score scoring the similarity of the

appearance of R_k in the first image and the appearance $S_{k,j}(R_k)$ in the second image;

bd selecting a transformation S_k from among the n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$ for which the similarity score of $S_k(R_k)$ is maximal among the similarity scores of the $S_{k,j}(R_k)$ for $j=1,\ldots n_k$;

be selecting a pixel $\mathbf{p}_k$ in R_k;

c defining a global transformation **T** mapping at least a portion of the pixel set into the pixel set based upon at least some of the $S_k(\mathbf{p}_k)$, the transformatioan T having an inverse $\mathbf{T}^{-1}$, the second image having an image under $\mathbf{T}^{-1}$; and

d obtaining the image of the second image under $\mathbf{T}^{-1}$, to produce a modified second image.

17. The program storage device of Claim 16 wherein the first and second separation patterns are one-dimensional separation patterns.

18. The program storage device of Claim 16 wherein the first and second separation patterns are two-dimensional separation patterns.

19. The program storage device of any one of the previous claims wherein the first and second separation patterns are obtained using a separation technique selected from the list comprising:

(a) electrophoresis;

(b) paper chromatography;

(c) thin-layer chromatography;

(d) high pressure liquid chromatography; and

(e) mass spectroscopy.

20. The program storage device of any one of the previous claims wherein the number of regions, m, is equal to one.

21. The program storage medium of any one of the previous claims wherein one or more of the regions R_k contain exactly one pixel.

22. The storage medium of any one of the previous claims wherein one or more of the regions R_k are rectangles.

23. The program storage device of any one of the previous claims wherein the regions R_k are rectangles that are regularly arranged in the first image.

24. The program storage device of any one of the previous claims wherein the step of dividing at least a portion of the pixel set into m regions R_k for k=1 to k=m comprises the steps of designating a scoring function scoring regions in the pixel set and selecting regions R_k for k=1 to m, where each region has a score greater than a predetermined value.

25. The program storage device of Claim 24 wherein the scoring function comprises the algorithmic expression $s=a[n]\cdot c$, wherein n is the number of spots in a region, c is the average contrast of the spots and $a[n]$ is a function of the number of spots in the region.

26. The program storage device of Claim 25 wherein $a[n]$ has a maximum at n approximately equal to 3.

27. The program storage device of any one of the previous claims wherein one or more of the transformations $S_{k,j}$ are of a type selected from the list comprising:

(a) translations;

(b) linear transformations; and

(c) affine transformations.

28. The program storage device of any one of the previous claims wherein the similarity score scoring the similarity of the appearance of R_k in the first image and the appearance of $S_{k,j}(R_k)$ in the second image is given by the arithmetic expression $\Sigma\ psf(\mathbf{x}, S_{k,j}(\mathbf{x}))$, wherein the sum is taken over all $\mathbf{x}$ in R_k, and $Psf(x,y) = 1+\alpha \min(B_1(x), B_2(y) + \beta\langle\nabla(f_1(x)), \nabla(f_2(y))\rangle^{1/2}$ for any two pixels $\mathbf{x}$ and $\mathbf{y}$ in the pixel set, wherein B_1 and B_2 are background contrast images of the first and second digital images, respectively, and α and β are predetermined constants.

29. The program storage device of any one of the previous claims wherein the global transformation is a spline.

30. The program storage device of any one of Claims 16 to 29 wherein the global transformation is a Delaunay triangulation transformation.

31. A computer program product comprising a computer useable medium having computer readable program code embodied therein for registering first and

second digital images of first and second separation patterns, respectively, the digital images being described by gray level functions f_1 and f_2, respectively, defined on a pixel set, the registering comprising the steps of:

a dividing at least a portion of the pixel set into m regions $R_1,\ldots,R_k,\ldots R_m$, the m regions each having an appearance in the first image;

b For each region R_k,

ba selecting a finite sequence of n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$, each transformation mapping R_k into the pixel set;

bb generating n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$ in the second digital image, n_k regions each having an appearance in the second image;

bc calculating a similarity score for each of the n_k regions $S_{k,j}(R_k)$ for $j=1,\ldots n_k$, the similarity score scoring the similarity of the appearance of R_k in the first image and the appearance of $S_{k,j}(R_k)$ in the second image;

bd selecting a transformation S_k from among the n_k transformations $S_{k,1},\ldots,S_{k,j},\ldots S_{k,n_k}$ for which the similarity score of $S_k(R_k)$ is maximal among the similarity scores of the $S_{k,j}(R_k)$ for $j=1,\ldots n_k$;

c defining a global transformation **T** mapping at least a portion of the pixels in the pixel set into the pixel set based upon at least some of the $S_k(\mathbf{p}_k)$, the transformation **T** having an inverse, the second digital image having an image under $\mathbf{T}^{-1}$; and

d obtaining the image of the second image under $\mathbf{T}^{-1}$, to produce a modified second image.

32. A method for registering a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising the steps of:

a registering the digital images I_1 and I_2 according to any one of Claims 1 to 15; to produce an image I'_2 that is the modified image of I_2;

b registering I'_k and I_{k+1}, for all k from 2 to n-1 according to any one of Claims 1 to 15 to produce an image I'_{k+1}, where I'_k and I'_{k+1} are the modified images of I_k and I_{k+1}, respectively.

33. A method for registering a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising registering a master pattern and the image I_k according to any one of Claims 1 to 15 so as to produce an image I'_k, where I'_k is the modified image of I_k, for all k from 1 to n.

34. A method for displaying a sequence of n digital images $I_n,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising:

a registering the sequence of images according to Claim 32, so as to produce a second sequence of images $I'_1, I'_2\ldots I'_n$, where I'_1 is the image I_1 and I'_k is the modified image of I_k, for k from 2 to n; and

b displaying the second sequence of images $I'_1,\ldots I'_n$ sequentially on a display.

35. A method for displaying a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising:

a registering the sequence of images according to Claim 33 so as to produce a second sequence of images $I'_1, I'_2,\ldots I'_n$, where I'_k is the modified image of I_k; and

b displaying the second sequence of images $I'_1,\ldots I'_n$ sequentially on a display.

36. A program storage device readable by machine, tangibly embodying a program of instructions executable by the machine to perform method steps for registering a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising the steps of:

a registering the digital images I_1 and I_2 according to any one of Claims 1 to 15; to produce an image I'_2 that is the modified image of I_2;

b registering I'_k and I_{k+1}, for all k from 2 to n-1 according to any one of Claims 1 to 15 to produce an image I'_{k+1}, where I'_k and I'_{k+1} are the modified images of I_k and I_{k+1}, respectively.

37. A computer program product comprising a computer useable medium having computer readable program code embodied therein for registering a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the computer program product comprising:

a computer readable program code for causing the computer to register the digital images I_1 and I_2 according to any one of Claims 1 to 15; to produce an image I'_2 that is the modified image of I_2;

b computer readable program code for causing the computer to register I'_k and I_{k+1}, for all k from 2 to n-1 according to any one of Claims 1 to 15 to produce an image I'_{k+1}, where I'_k and I'_{k+1} are the modified images of I_k and I_{k+1}, respectively.

38. A program storage device readable by machine, tangibly embodying a program of instructions executable by the machine to perform method steps for registering a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the method comprising registering a master pattern and the image I_k according to any one of Claims 1 to 15 so as to produce an image I'_k, where I'_k is the modified image of I_k, for all k from 1 to n.

39. A program storage device readable by machine, tangibly embodying a program of instructions executable by the machine to perform method steps for displaying a sequence of n digital images $I_1,\ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set. the method comprising:

a registering the sequence of images according to Claim 32, so as to produce a second sequence of images I'_1, $I'_2\ldots I'_n$, where I'_1 is the image I_1 and I'_k is the modified image of I_k, for k from 2 to n; and

b displaying the second sequence of images $I'_1,\ldots I'_n$ sequentially on a display.

40. A computer program product comprising a computer useable medium having computer readable program code embodied therein for displaying a sequence of n digital images $I_n, \ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the computer program product comprising:

a computer readable program code for causing the computer to register the sequence of images according to Claim 32, so as to produce a second sequence of images $I'_1, I'_2 \ldots I'_n$, where I'_1 is the image I_1 and I'_k is the modified image of I_k, for k from 2 to n; and

b computer readable program code for causing the computer to display the second sequence of images $I'_1, \ldots I'_n$ sequentially on a display.

41. A program storage device readable by machine, tangibly embodying a program of instructions executable by the machine to perform method steps for displaying a sequence of n digital images $I_n, \ldots I_n$ of n separation patterns, respectively. the digital images being described by gray level functions defined on a pixel set, the method comprising:

a registering the sequence of images according to Claim 33 so as to produce a second sequence of images $I'_1, I'_2, \ldots I'_n$, where I'_k is the modified image of I_k; and

b displaying the second sequence of images $I'_1, \ldots I'_n$ sequentially on a display.

42. A computer program product comprising a computer useable medium having computer readable program code embodied therein for displaying a sequence of n digital images $I_n, \ldots I_n$ of n separation patterns, respectively, the digital images being described by gray level functions defined on a pixel set, the computer program product comprising:

a computer readable program code for causing the computer to registering the sequence of images according to Claim 33 so as to produce a second sequence of images $I'_1, I'_2, \ldots I'_n$, where I'_k is the modified image of I_k; and

b computer readable program code for causing the computer to displaying the second sequence of images $I'_1,\ldots I'_n$ sequentially on a display.

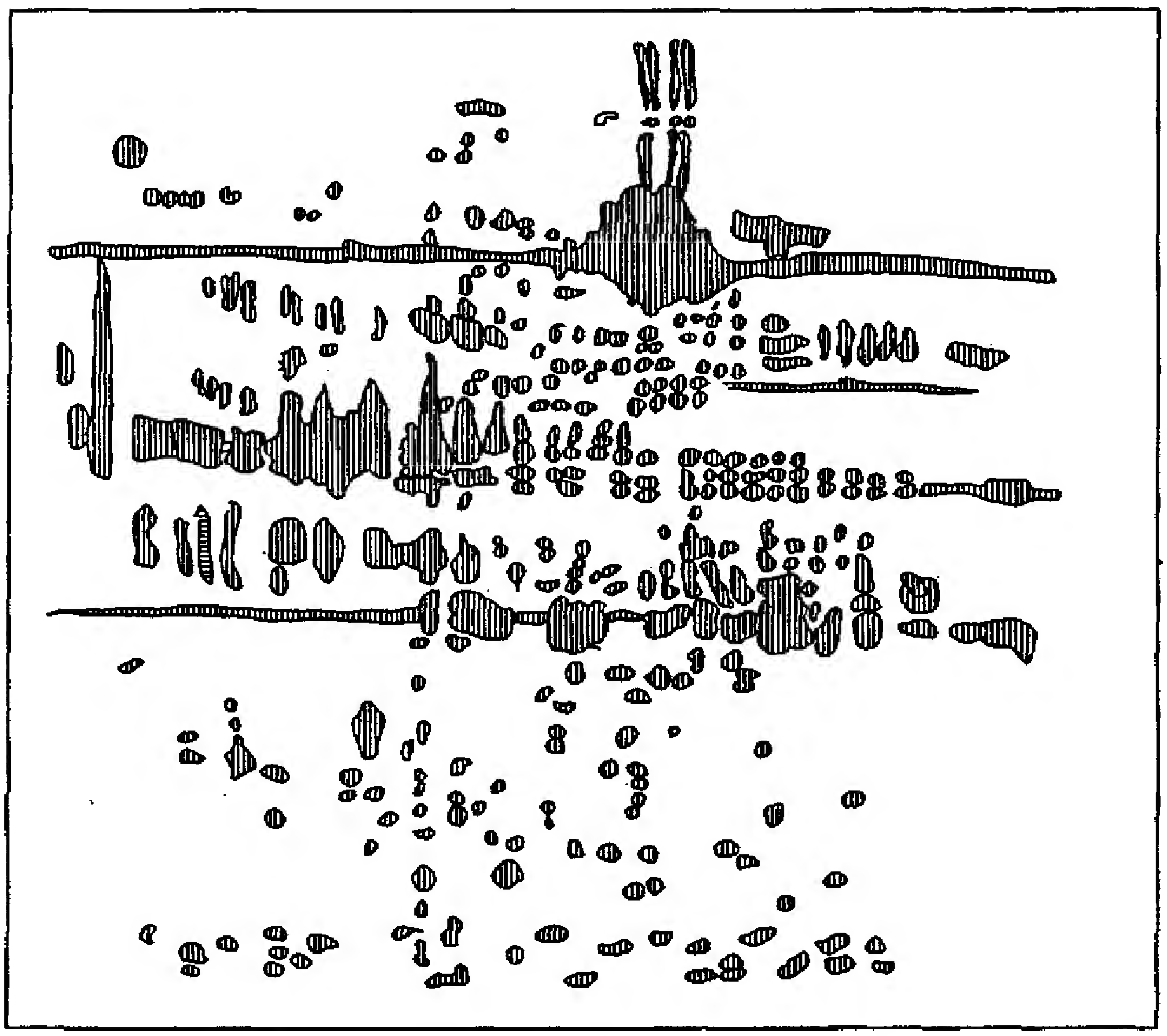

FIG. 1A

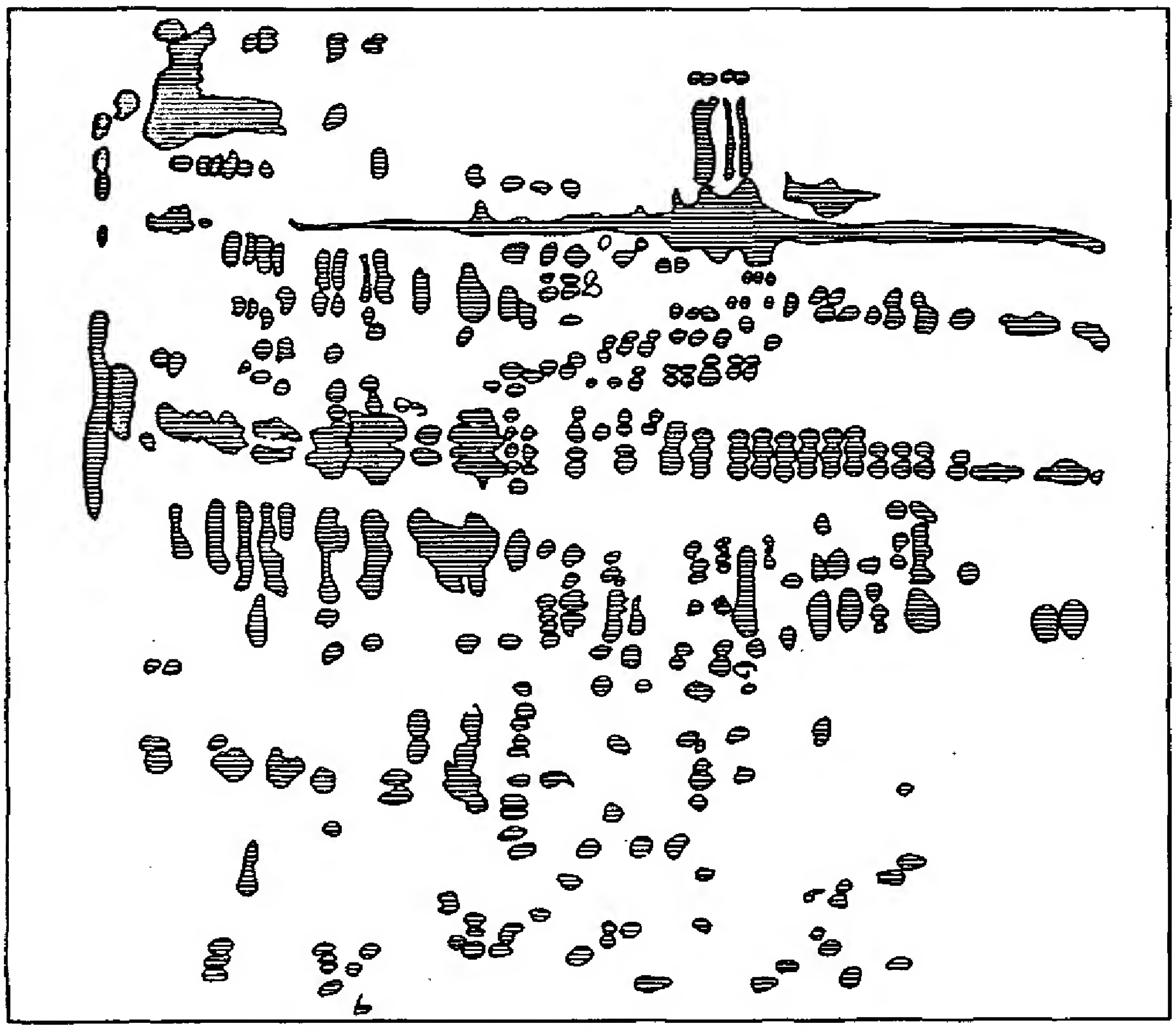

FIG. 1B

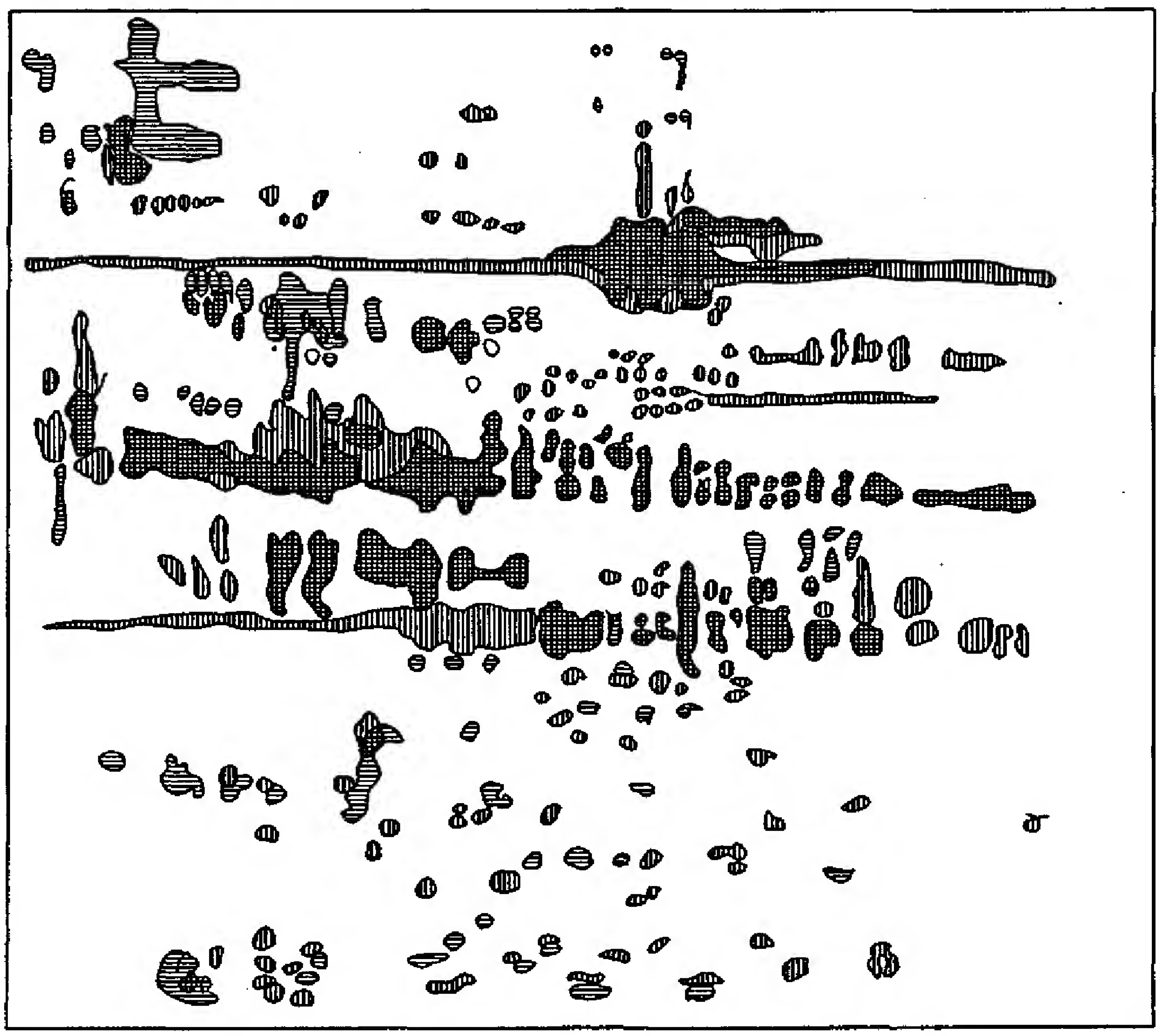

FIG. 1C

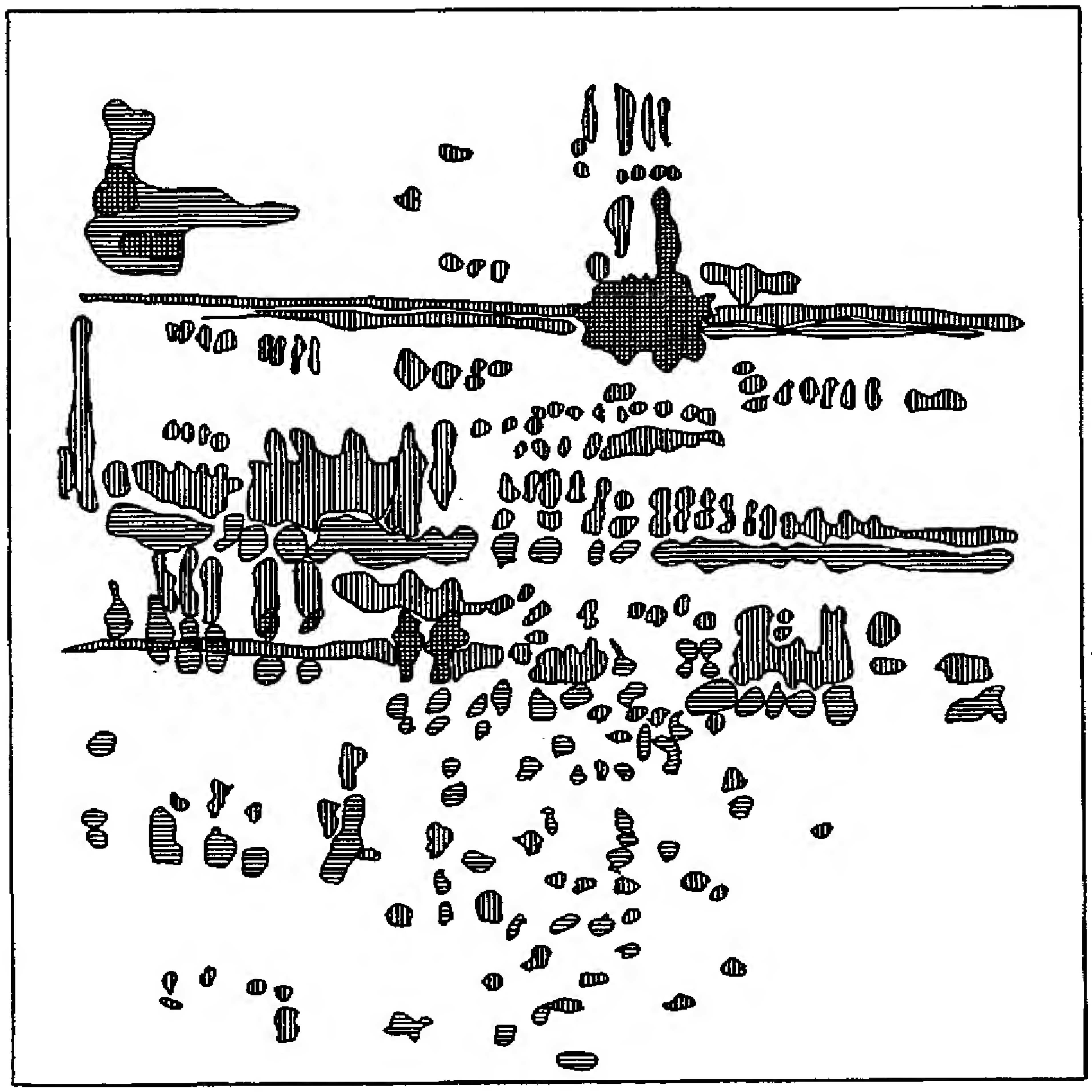

FIG. 1D

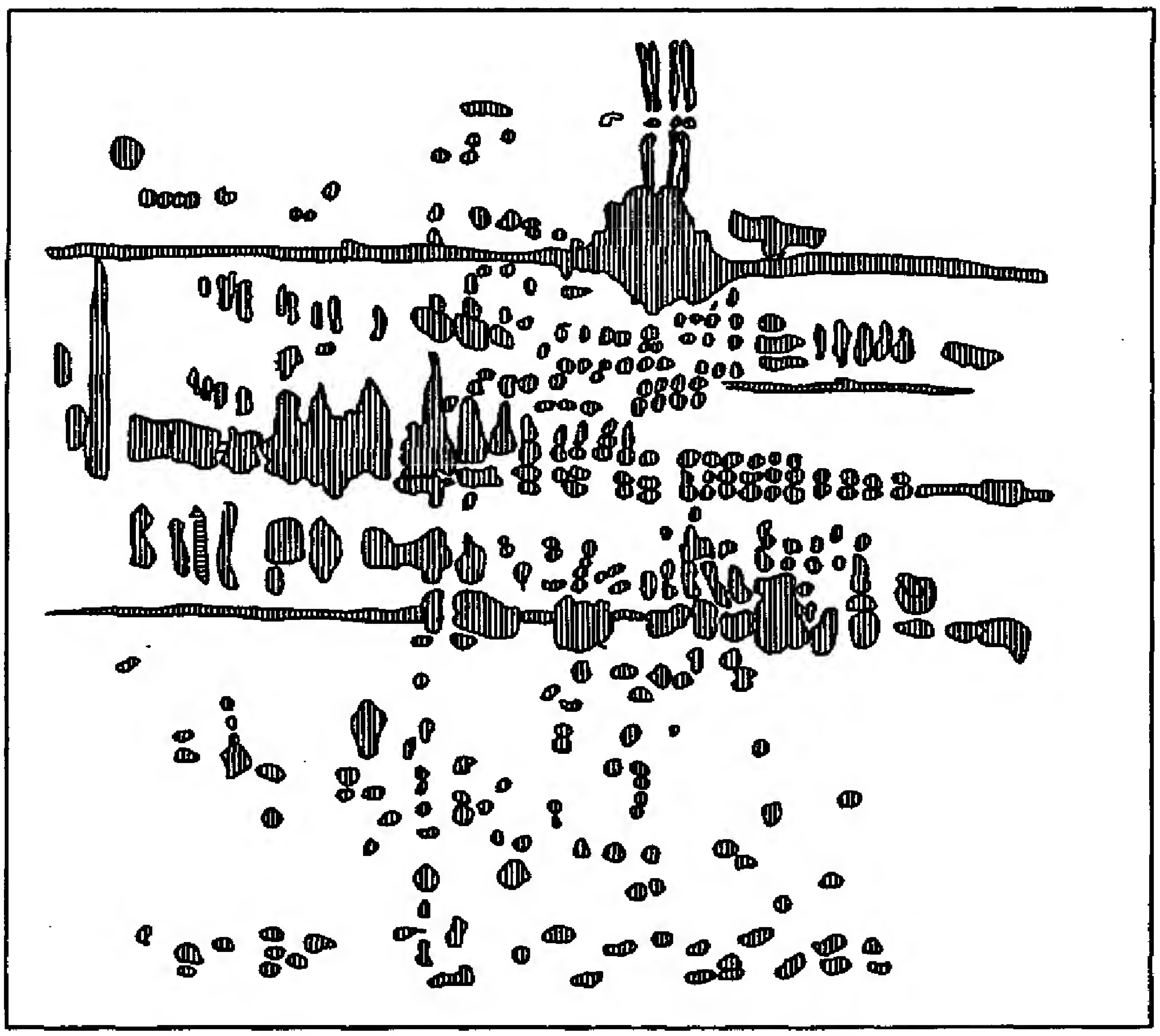

FIG. 2A

FIG. 2B

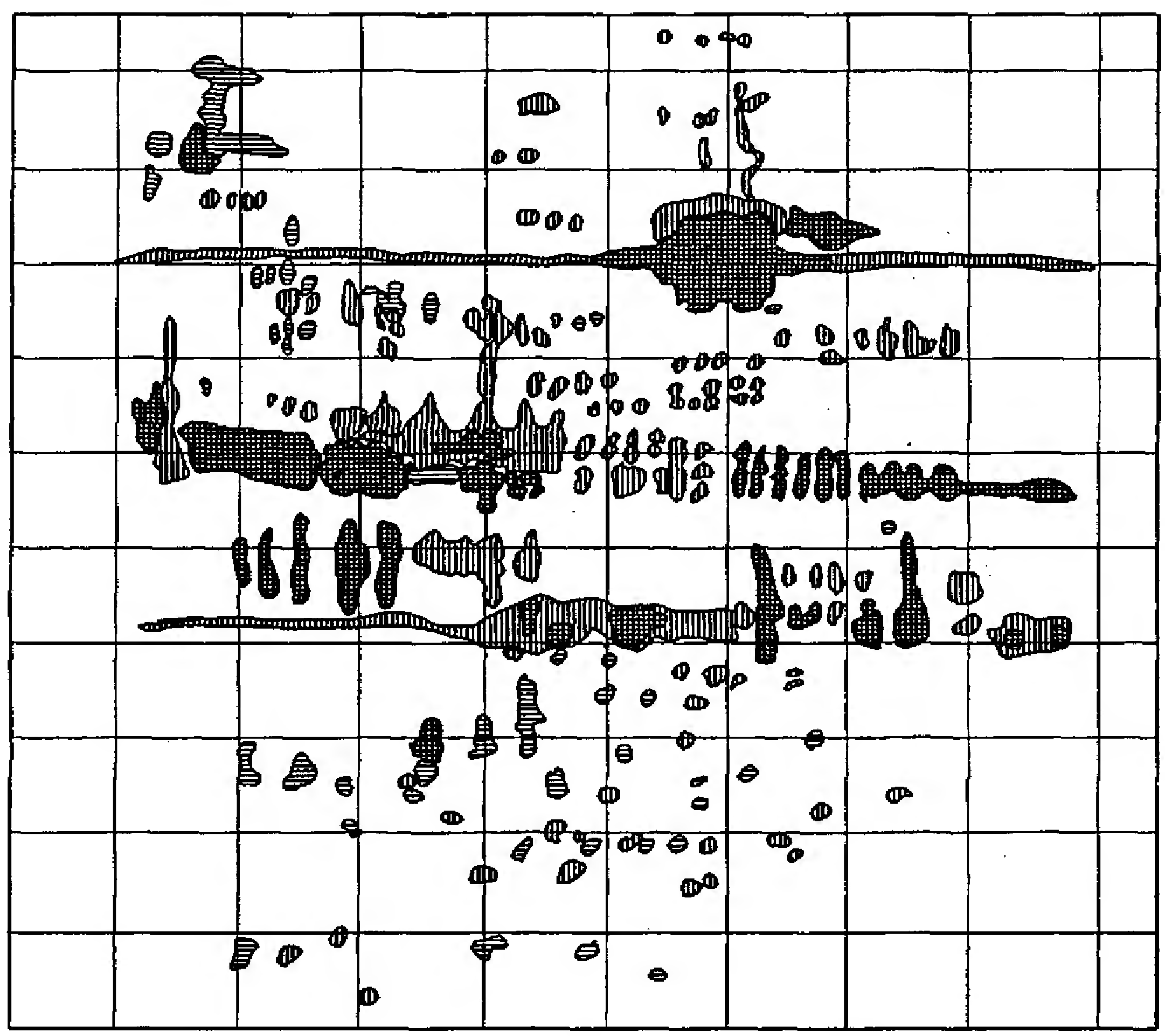

FIG. 2C

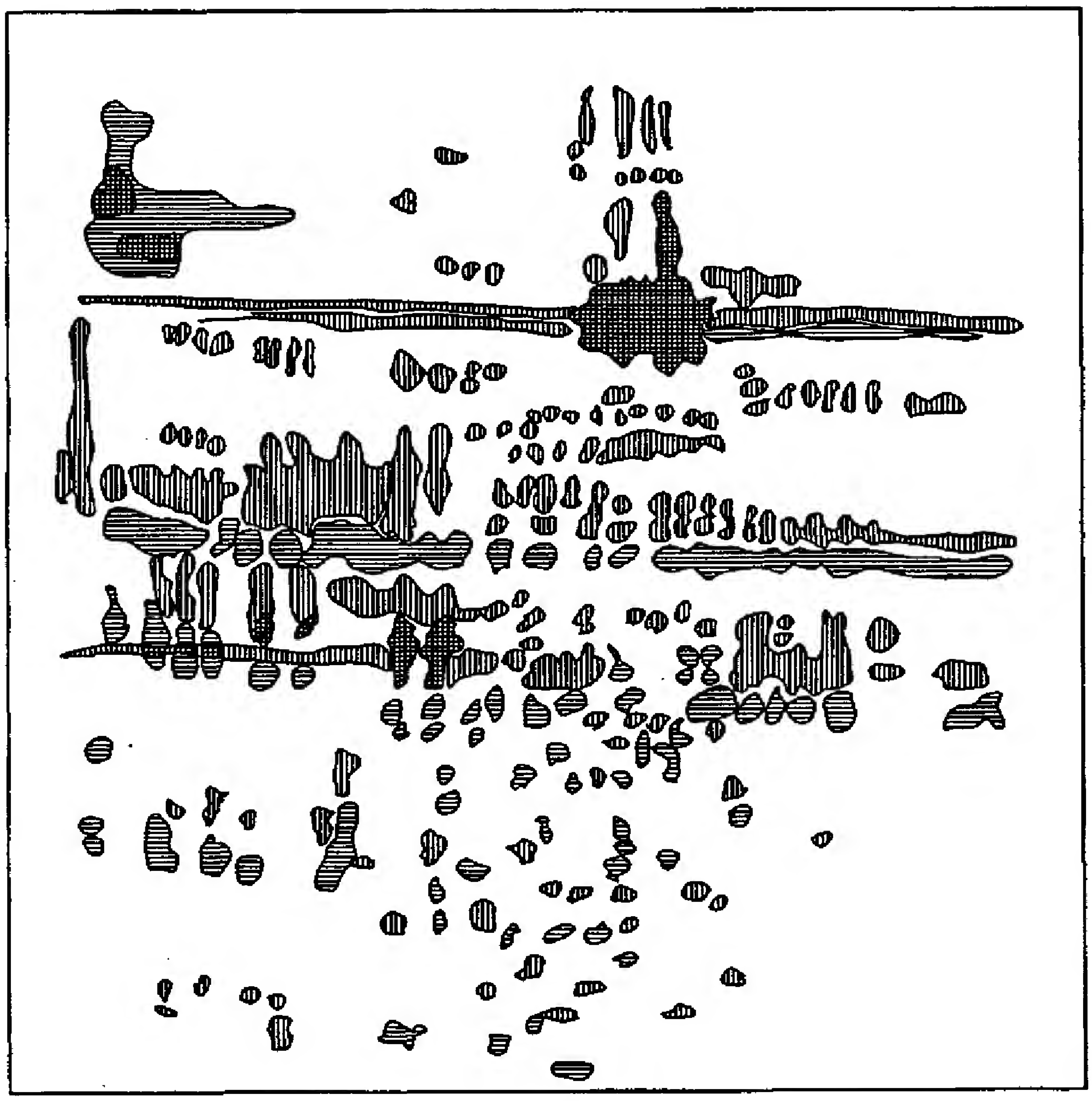

FIG. 2D

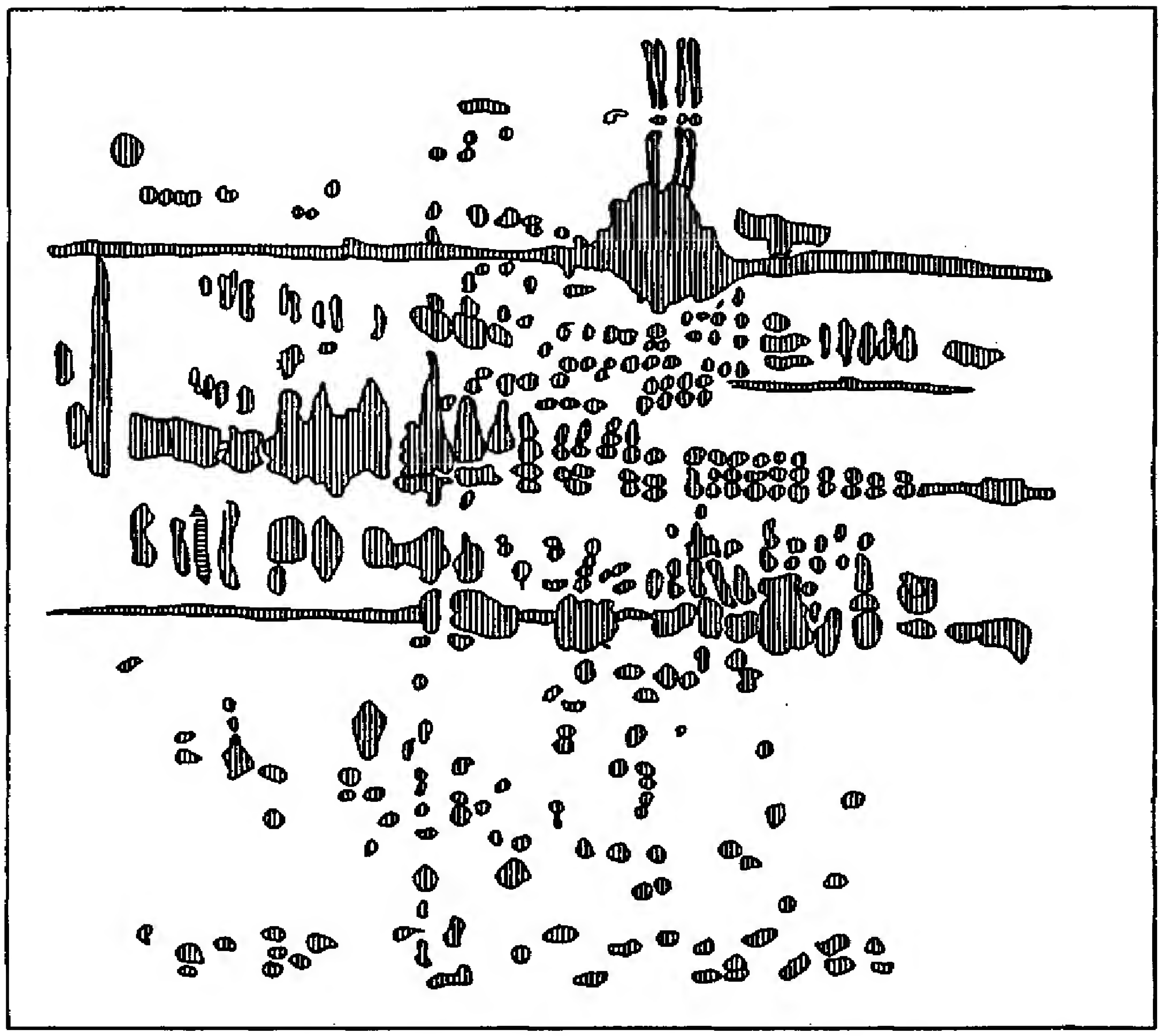

FIG. 3A

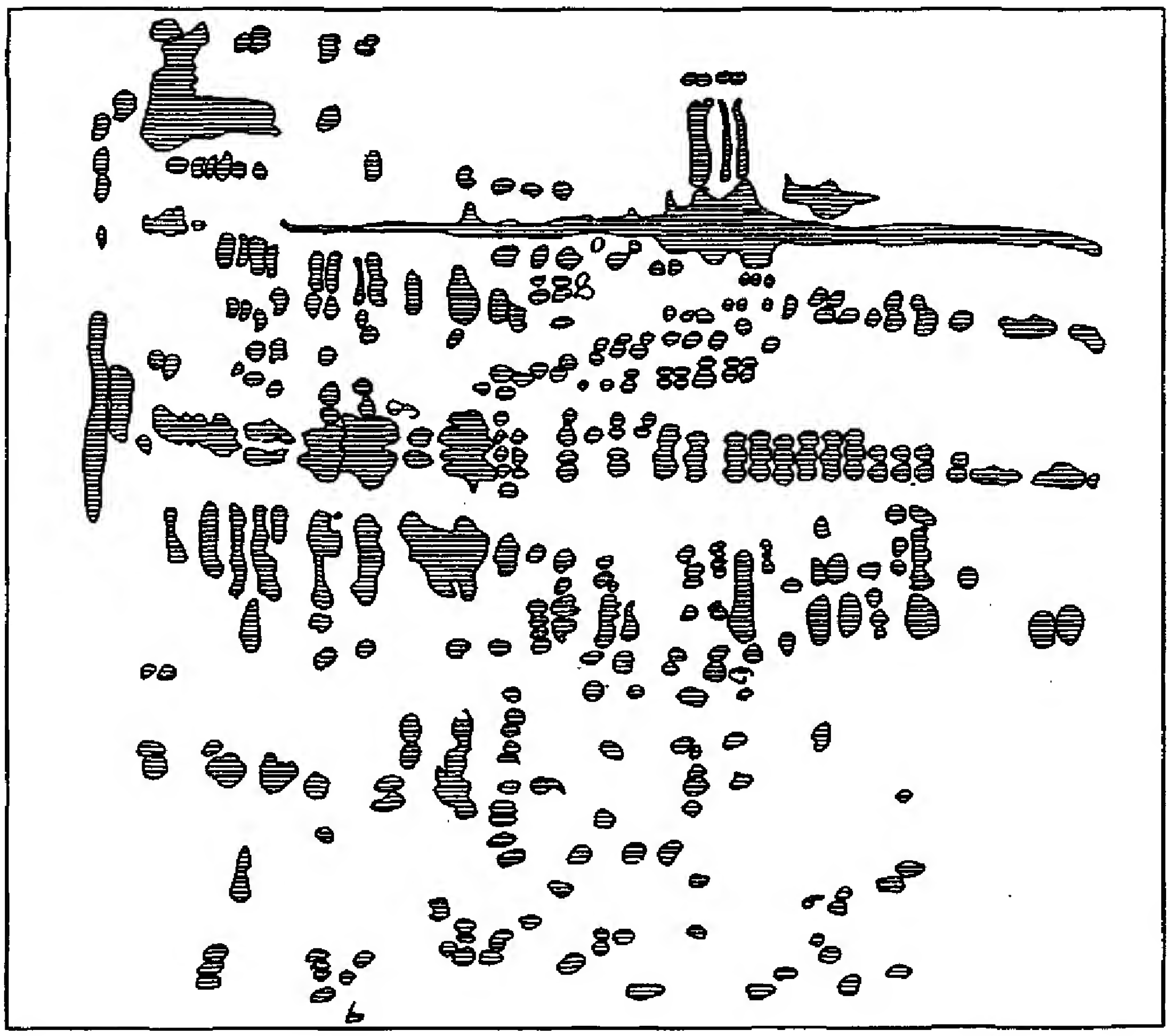

FIG. 3B

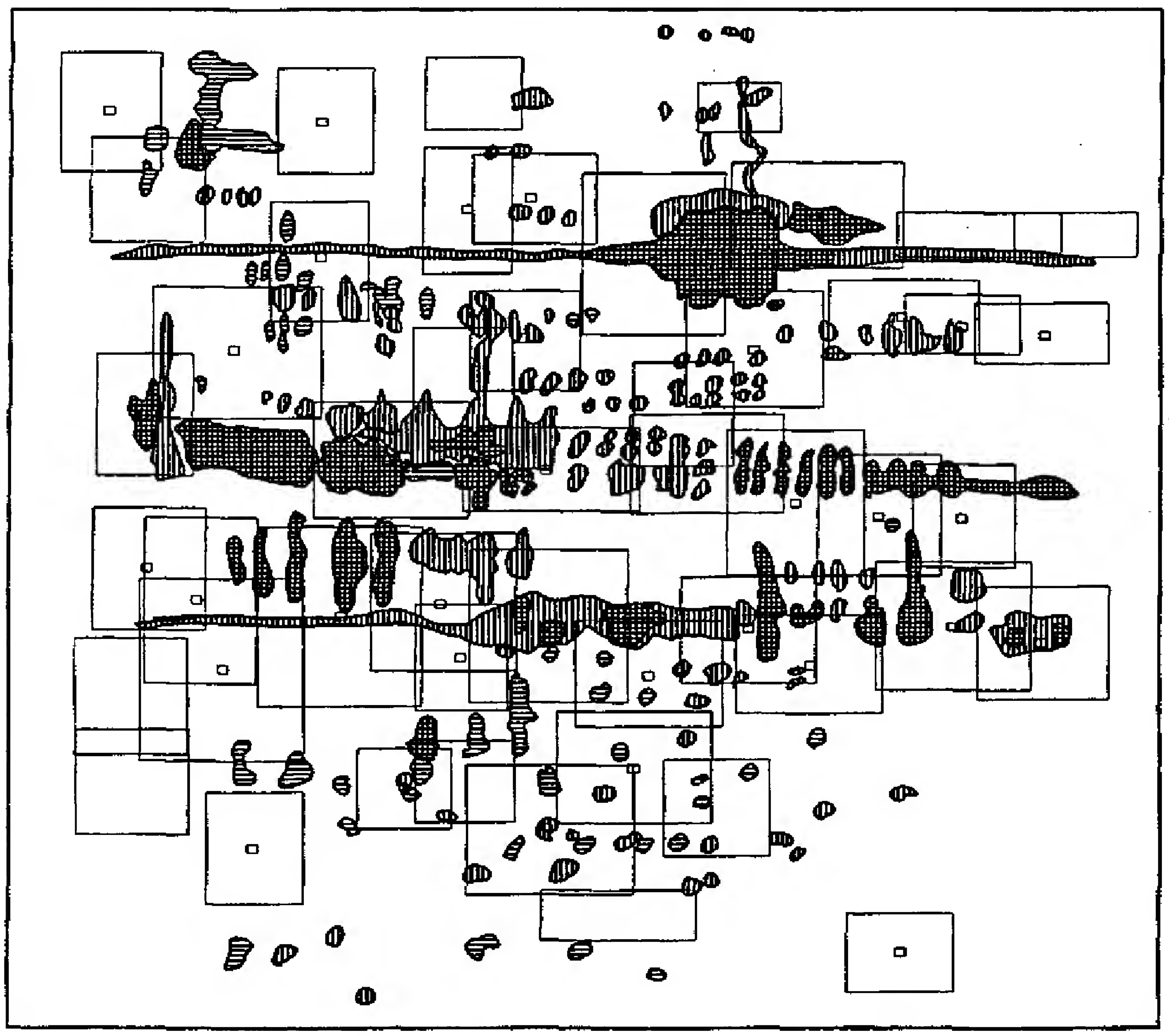

FIG. 3C

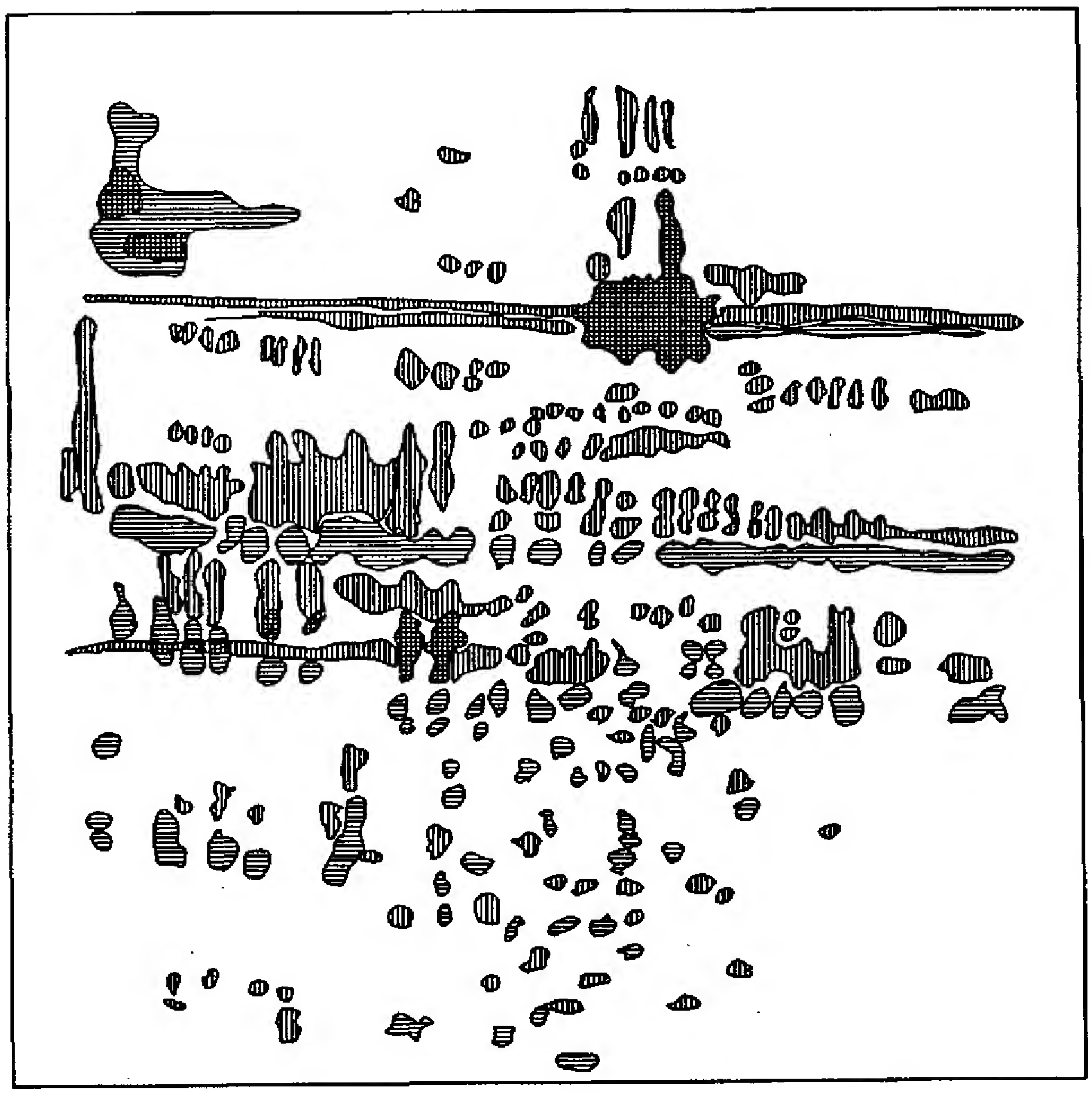

FIG. 3D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 00/00778

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G06T7/00 G06K9/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G06T G06K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

INSPEC, WPI Data, IBM-TDB, PAJ, EPO-Internal, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BASRI R ET AL: "RECOGNITION USING REGION CORRESPONDENCES" INTERNATIONAL JOURNAL OF COMPUTER VISION,US,KLUWER ACADEMIC PUBLISHERS, NORWELL, vol. 25, no. 2, 1 November 1997 (1997-11-01), pages 145-166, XP000723757 ISSN: 0920-5691 page 147, right-hand column, paragraph 2 page 148, left-hand column, paragraph 2 p. 148-9 Section "2. Problem Definition" --- -/--	1-40

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2001	07/05/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Granger, B

2

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	FLUSSER J ET AL: "A MOMENT-BASED APPROACH TO REGISTRATION OF IMAGES WITH AFFINE GEOMETRIC DISTORTION" IEEE TRANSACTIONS ON GEOSCIENCE AND REMOTE SENSING,US,IEEE INC. NEW YORK, vol. 32, no. 2, 1 March 1994 (1994-03-01), pages 382-387, XP000456930 ISSN: 0196-2892 abstract ---	1-40
A	WO 98 50885 A (SARNOFF CORP) 12 November 1998 (1998-11-12) claim 8 ---	1-40
A	US 5 073 963 A (KO WEN-JENG ET AL) 17 December 1991 (1991-12-17) cited in the application abstract -----	1-40

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 00/00778

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9850885 A	12-11-1998	EP 1016038 A	05-07-2000
US 5073963 A	17-12-1991	AU 8001991 A	31-12-1991
		WO 9119274 A	12-12-1991

Form PCT/ISA/210 (patent family annex) (July 1992)